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# The Underestimated Potential of Organometallic Rhenium Complexes as Anticancer Agents

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**ABSTRACT:** In the recent years, organometallic compounds have become recognized as promising anti-cancer drug candidates. While radioactive  $^{186/188}\text{Re}$  compounds are already used in clinics for cancer treatment, cold Re organometallic compounds have mostly been explored as luminescent probes and photosensitizers in photo-catalysis. However, a growing number of studies have recently revealed the potential of Re organometallic complexes as anticancer agents. Several compounds have displayed cytotoxicity equaling or exceeding that of the well-established anti-cancer drug cisplatin. In this review, we present the currently known Re organometallic complexes that have shown anti-proliferative activity on cancer cell lines. A particular emphasis is placed on their cellular uptake and localization as well as their potential mechanism of action.

## INTRODUCTION

Despite the growing body of research, cancer remains among the leading causes of death in the world, in particular in high-income countries (although cancer rates are increasing in lower-income countries which already account for 70% of world cancer deaths).<sup>1</sup> The survival rates hugely vary with the type and stage of tumor, as well as age, sex and socio-economic situation of each patient.<sup>1,2</sup> For example, about 50% of adult (15-99 years old) patients diagnosed with cancer in England and Wales in 2010-2011 are expected to survive for another 10 years or more.<sup>2</sup>

However, while patients with testicular cancer, melanoma, prostate cancer and Hodgkin lymphoma have a ten-year survival rate of over 80%, less than 15% of patients with pancreas, lung, brain, esophagus and stomach cancers survive that long.<sup>2</sup> Cancer treatment depends on the type, stage and location of tumor, but most patients undergo chemotherapy either as the main treatment or in combination with surgery and/or radiotherapy. Over 50% of tumors are treated with platinum-based drugs (cisplatin, carboplatin, oxaliplatin) alone or co-administered with other chemotherapeutic agents.<sup>3</sup> Nevertheless, new anti-cancer drugs are still being actively sought due to the emergence of platinum resistance and the severe side effects associated with the chemotherapeutic treatments.<sup>4</sup> In the last decades, many organometallic compounds (i.e. compounds containing at least one metal-carbon bond) have proven themselves as extremely promising anti-cancer drug candidates.<sup>5-14</sup> Organometallic complexes offer structural and stereochemical variety,<sup>15, 16</sup> the possibility of rational ligand design, and diverse mechanisms of action (e.g. *via* redox activity, ligand exchange, catalytic activity and/or photo-activity).<sup>17-21</sup>

Compared with some intensively investigated organometallic complexes such as those based on ruthenium,<sup>12, 13</sup> relatively few examples of cytotoxic rhenium organometallic compounds are found in the literature. However, numerous studies have been recently reported describing the potential of such compounds.<sup>22-47</sup> Indeed, Re organometallic compounds possess several intrinsic properties advantageous for the development of novel anti-cancer drug candidates. For instance, many Re organometallic complexes are excellent luminescent probes with long-lived emission states, large Stokes shifts, high quantum yields and emission that can be tuned by varying the ligands.<sup>37, 48</sup> They can thus be easily followed inside cells by conventional and/or time-resolved (e.g. FLIMS) emission microscopy to elucidate their cellular distribution and mechanism of action. Furthermore, all reported cytotoxic Re organometallic compounds are based on the  $\text{Re}(\text{CO})_3$  core, which is chemically robust and easily accessible, e.g. from  $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+ / [\text{ReBr}_3(\text{CO})_3]^{2-}$ ,<sup>29, 34</sup>  $\text{Re}(\text{CO})_5\text{Cl} / \text{Re}(\text{CO})_5\text{Br}$ <sup>26-28, 31-33, 41, 42, 49</sup> or  $\text{Re}_2(\text{CO})_{10}$ ,<sup>24, 30, 40</sup> in 1-3 step synthesis. The introduced ligands can be designed to incorporate other functional moieties such as targeting biomolecules.<sup>28, 29, 34, 35, 39, 41, 44, 46, 47, 50, 51</sup> However, the key property of the Re organometallic complexes is the possibility to prepare “hot” analogues that can be applied

for radio-imaging as well as for therapy. Besides  $^{186}\text{Re}$  and  $^{188}\text{Re}$  isotopes,  $^{99\text{m}}\text{Tc}$  can often be used with the same set of ligands as the cold Re due to the similarity in the coordination chemistry of these group 7 congeners.<sup>52-55</sup> Thus, a Re complex has the potential to be a multi-modal molecule, which acts as a luminescent probe, a radio-imaging and a pharmaceutical agent. Importantly, “hot” analogues can be used to run *in vivo* biodistribution and pharmacokinetics studies, which are crucial for the development of new drugs.

In this review, we present the cytotoxic Re organometallic complexes that have been reported in the literature to date. Their modes of cytotoxic action, when known, are discussed in detail. The compounds presented in this review are categorized into two main groups according to the nature of their anti-proliferative activity, namely “traditional” cytotoxicity and photo-toxicity. Anti-cancer and other therapeutic applications of radio-pharmaceuticals based on  $^{186/188}\text{Re}$  have been extensively described in several excellent reviews.<sup>54, 56-59</sup> Since some of them are extremely recent, this topic is not discussed herein. Of note, two relatively recent and comprehensive reviews by Lo *et al.*<sup>37</sup> and Coogan *et al.*<sup>48</sup> have discussed the cellular uptake, localization and cytotoxicity of luminescent Re(I) organometallic complexes, but both are more focused on imaging applications and do not cover non luminescent organometallic Re compounds. As can be seen in these reviews, several Re complexes have been reported to interact with DNA and/or proteins, yet not all of them have been screened for cytotoxicity. Here we discuss only the Re organometallic compounds that have been studied in cell culture.

## CYTOTOXIC Re ORGANOMETALLIC COMPLEXES

**Re(I) tricarbonyl bisimine complexes.** A large percentage of cytotoxic Re organometallic compounds have been initially developed as luminescent probes and their anti-cancer effect has only been discovered during biocompatibility screenings. Unsurprisingly, the largest family of anti-proliferative Re organometallic compounds is that of [2+1] Re(I) tricarbonyl bisimine complexes  $[\text{Re}(\text{CO})_3(\text{bisimine})\text{L}]$  where L is a monodentate pyridine derivative or a halide (Cl, Br) ligand. These compounds normally possess outstanding photochemical properties and their bisimine or pyridine ligands can be easily derivatized. Typically used bisimine ligands are 2,2'-

bipyridine or phenantroline-based (Figure 1) and, despite apparent similarity, cytotoxicity varies considerably from one derivative to another.

The cytotoxicity of several bipyridine and phenantroline based Re(I) complexes (Figure 1A and B, **1-14**) have been reported. Their anti-proliferative activity reached or even exceeded the one of cisplatin on various human cancer cell lines.<sup>28, 30, 32, 39, 40, 45, 49, 51</sup> The cytotoxicity of the compounds generally increased with lipophilicity,<sup>32, 33, 39, 49, 51</sup> most probably due to an improved cellular uptake. Re(I) complexes **9** (Figure 1A) derivatized with a fluoros pendant were the only exception to this trend. Their cytotoxicity diminished with rising lipophilicity.<sup>39</sup>

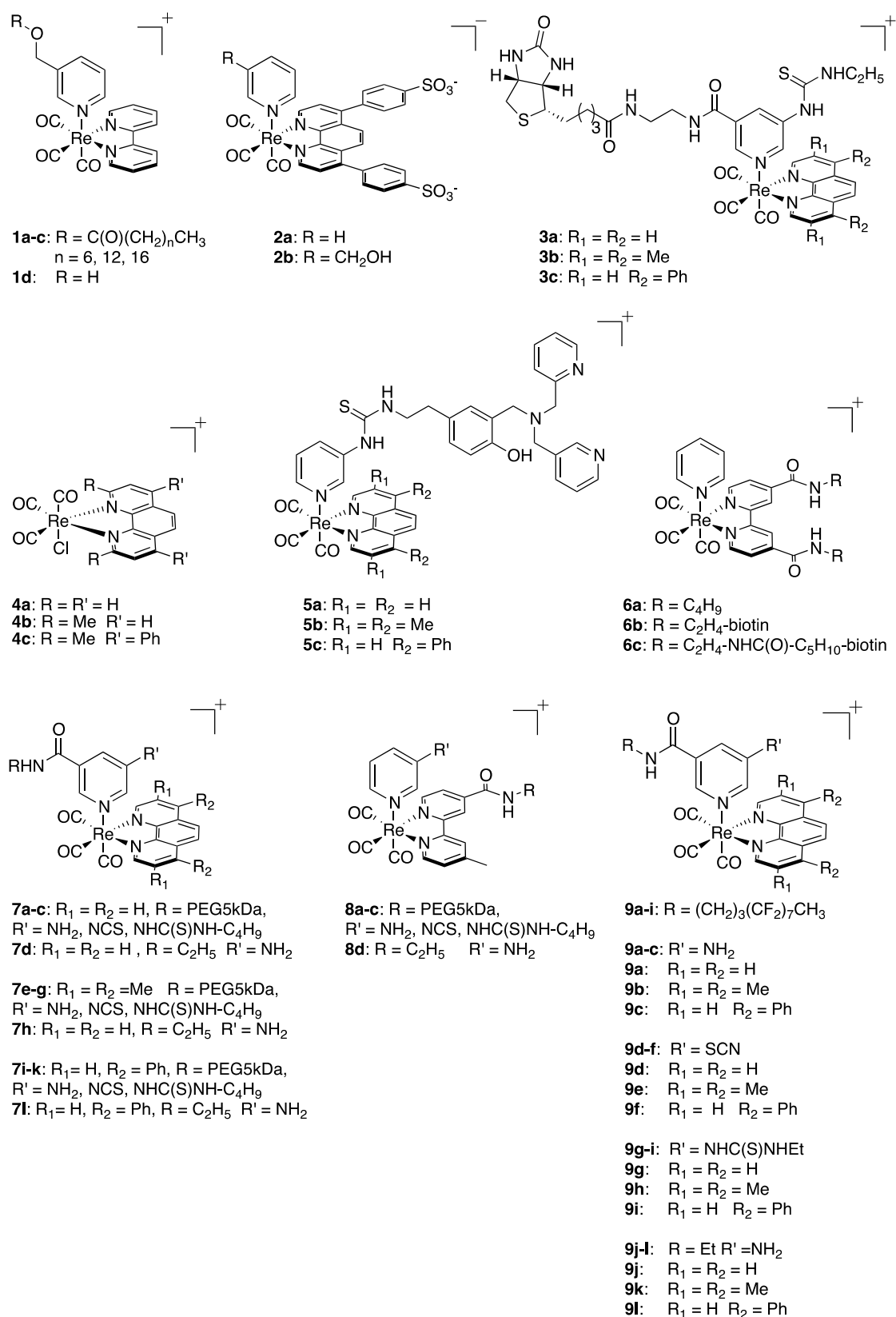
However, these compounds are exceptionally lipophilic ( $\log P_{o/w}$  in the range of 5 to 9) and could form self-aggregates due to favorable F-F interactions. The uptake of anti-proliferative Re(I) tricarbonyl bisimine complexes is often very fast and efficient, yet little is known about their mechanism. Some cytotoxic cationic complexes such as **1a-c** appear to accumulate in cells *via* passive diffusion.<sup>49</sup> Re luminescent probes used to sense Cd(II) and Zn(II) (**5** on Figure 1A) are, however, taken up *via* an energy-dependent pathway such as endocytosis as demonstrated by temperature and ATP production inhibition uptake assays.<sup>32</sup> Interestingly, fluorescence microscopy cellular localization studies have never – to the best of our knowledge – identified a cytotoxic Re(I) tricarbonyl bisimine complex located inside the nucleus. All compounds screened for cytotoxicity were indeed imaged in the cytoplasm.<sup>28, 32, 33, 39, 51</sup> When co-localization experiments were performed on Re organometallic complexes **7i** and **9f** (Figure 1A), predominately mitochondrial (up to 80%) localization was reported.<sup>39</sup> A non-negligible percentage of endosomal accumulation (about 20%) has also been detected for compound **7i**.<sup>39</sup> As a note of caution, although fluorescent microscopy imaging is an important tool in the investigation of the cellular behavior of Re organometallic complexes, their luminescence can sometimes be quenched in cells.<sup>39, 44, 47</sup> Due to this problem, direct measurements of Re content by inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS)<sup>60</sup> can be a more accurate method to quantify the cellular uptake and distribution of Re complexes.

The first cytotoxic bisimine Re complex (**15** on Figure 1B) with anti-proliferative properties, however, was in some ways an exception to the typical photo-physical and biological properties of these compounds. Based on a pyridine-triazine ligand (2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine), compound **15** emitted only in the solid state and was designed in a crescent shape to fit the major or minor grooves of DNA.<sup>27</sup> Experimental evaluation of its interactions with DNA has indeed demonstrated that **15** was non-intercalatively binding to DNA with a preference for AT-rich sequences. Modeling studies suggested the minor groove as binding site. The resulting cytotoxicity was only moderate (IC<sub>50</sub> of 30-50  $\mu$ M compared with 10-20  $\mu$ M IC<sub>50</sub> for cisplatin) on KB-3-1 (human epidermoid carcinoma), HepG2 (human liver hepatocellular carcinoma) and HeLa (human cervical cancer) and low (195  $\mu$ M compared with 40  $\mu$ M for cisplatin) on human multi-drug resistant epidermal carcinoma KB-V-1 cell line. Since the compound was not fluorescent in solution, its localization in cell could not be determined by fluorescence microscopy. Therefore it is unknown whether it reaches cell nucleus or/and mitochondria where it could interact with DNA.

Another Re(I) tricarbonyl diimine compound using neither bipyridine nor phenanthroline, but asymmetric azines (**16** on Figure 1B) as bidentate ligands have been reported to be active on human neuroblastoma NB69, glioblastoma astrocytoma U373, breast cancer MRC-7 (hormone-responsive) and EVSA-T (hormone-independent) with IC<sub>50</sub> in the 4–10  $\mu$ M range.<sup>31</sup> Some azines are known to possess some anti-cancer activity on their own, but their cytotoxicity was increased by coordination to the Re center. Curiously, Re(CO)<sub>5</sub>Cl displayed the same activity as Re(I) azines, while Re(CO)<sub>5</sub>Br failed to produce a strong anti-proliferative effect on U2OS (osteosarcoma, >100  $\mu$ M), HepG2 (>100  $\mu$ M) and MCF-7 (57  $\mu$ M) in a different study.<sup>44</sup>

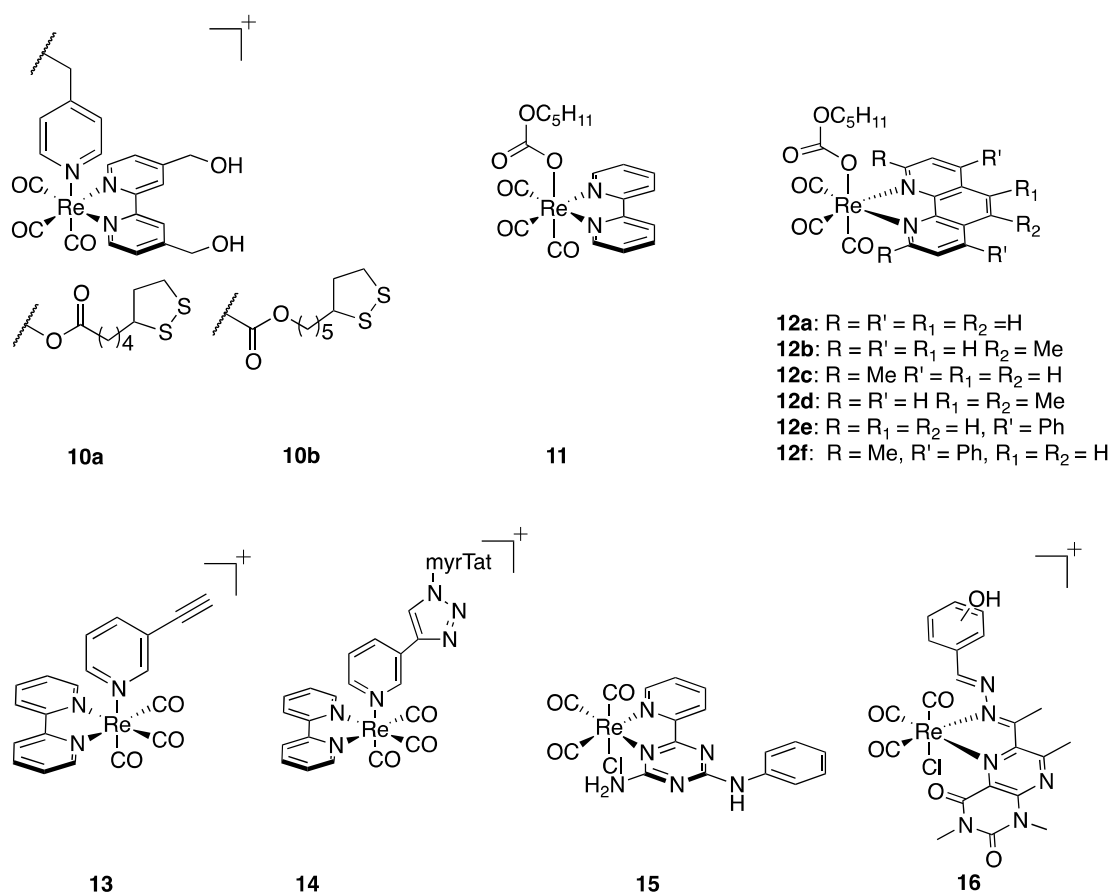
Although a substantial number of Re(I) tricarbonyl bisimine complexes [Re(CO)<sub>3</sub>(bisimine)L] have been reported, their mechanism of cytotoxicity has not yet been fully elucidated. In particular, studies on the compounds' stability in biological conditions (e.g. in blood plasma) are lacking. Evaluation of [Re(CO)<sub>3</sub>(bisimine)L] complexes' stability could shed some light on their behavior in cells, since, for some of these compounds, the monodentate ligand

can be easily substituted<sup>51, 61-64</sup> or modified<sup>40</sup> under physiological conditions. For instance, the monodentate pyridine ligand can sometimes be substituted by stronger donor groups (e.g. histidine or cysteine),<sup>61</sup> while alkylcarbonato ligands (compounds **11** and **12** on Figure 1B) are transformed into alkyloxo *via* CO<sub>2</sub> loss in cell culture medium.<sup>40</sup> In fact, [2+1] <sup>99m</sup>Tc tricarbonyl pyridine-based complexes have also been prepared, but have not been extensively developed due to concerns about their stability.<sup>61-63</sup>



**Figure 1A.** Cytotoxic Re(I) tricarbonyl bisimine complexes.





**Figure 1B.** Cytotoxic Re(I) tricarbonyl bisimine complexes (continued).

**Table 1.** Cytotoxicity of Re organometallic compounds towards human cancer cell lines.

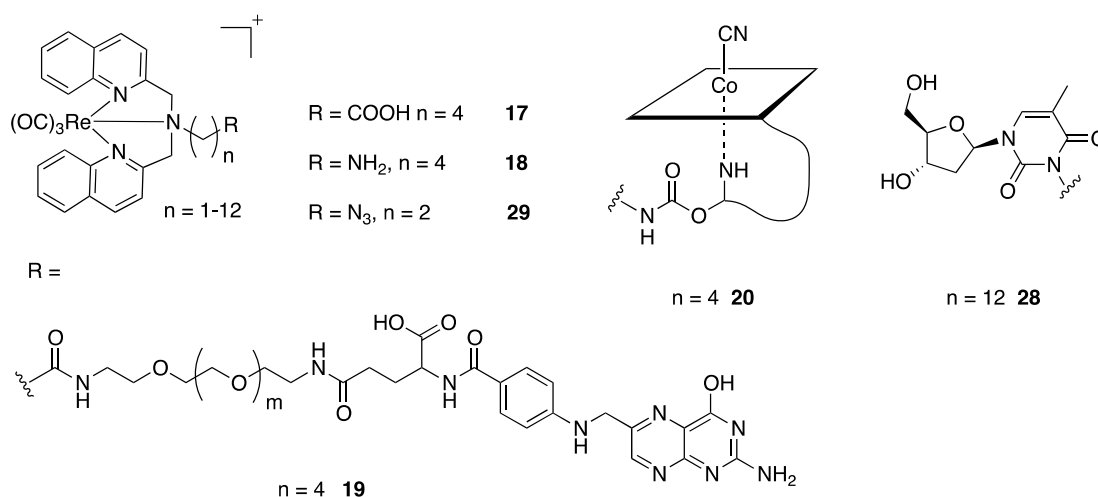
Compound	IC <sub>50</sub> (μM)	Known drug <sup>a</sup> IC <sub>50</sub> (μM)	Cell line	Incubation time	Reference
<b>3a</b>	22.7	26.7	HeLa	48 h	28
<b>3b</b>	17.5	26.7	HeLa	48 h	28
<b>3c</b>	28.5	26.7	HeLa	48 h	28
<b>5a</b>	4.0 ± 0.5	19 ± 0.4	HeLa	48 h	32
<b>5b</b>	2.3 ± 0.2	19 ± 0.4	HeLa	48 h	32
<b>5c</b>	0.7 ± 0.1	19 ± 0.4	HeLa	48 h	32
<b>6a</b>	7.7 ± 1.0	25.6 ± 2.3	HeLa	48 h	33
<b>7a</b>	26.0 ± 1.6	11.9 ± 0.3	HeLa	48 h	39
<b>7d</b>	15.0 ± 4.8	11.9 ± 0.3	HeLa	48 h	39
<b>7e</b>	11.9 ± 1.6	11.9 ± 0.3	HeLa	48 h	39
<b>7h</b>	5.0 ± 0.4	11.9 ± 0.3	HeLa	48 h	39
<b>7i</b>	6.6 ± 0.4	11.9 ± 0.3	HeLa	48 h	39
<b>7l</b>	3.6 ± 0.4	11.9 ± 0.3	HeLa	48 h	39
<b>8a</b>	>1151.7	11.9 ± 0.3	HeLa	48 h	39
<b>8d</b>	159.1 ± 8.0	11.9 ± 0.3	HeLa	48 h	39
<b>9a</b>	4.01 ± 0.14	34.0 ± 3.3	HeLa	48 h	65
<b>9b</b>	6.10 ± 0.37	34.0 ± 3.3	HeLa	48 h	65
<b>9c</b>	10.7 ± 0.53	34.0 ± 3.3	HeLa	48 h	65
<b>9g</b>	12.9 ± 0.23	34.0 ± 3.3	HeLa	48 h	65
<b>9h</b>	16.6 ± 0.36	34.0 ± 3.3	HeLa	48 h	65
<b>9i</b>	39.9 ± 1.76	34.0 ± 3.3	HeLa	48 h	65
<b>9j</b>	15.0 ± 4.8	34.0 ± 3.3	HeLa	48 h	65
<b>9k</b>	5.0 ± 0.4	34.0 ± 3.3	HeLa	48 h	65
<b>9l</b>	3.6 ± 3.3	34.0 ± 3.3	HeLa	48 h	65
<b>11</b>	3.0 ± 2.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>12a</b>	2.0 ± 1.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>12b</b>	3.0 ± 1.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>12c</b>	2.0 ± 2.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>12d</b>	3.0 ± 4.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>12e</b>	4.0 ± 6.5	5.0 ± 2.5	MDA-MB-468	48 h	45
<b>13</b>	29.9 ± 6.1	9.1 ± 2.8	HeLa	48 h	51
<b>14</b>	13.0 ± 2.8	9.1 ± 2.8	HeLa	48 h	45
<b>15</b>	43 ± 2.2	22.1 ± 3.6	KB-3-1	48 h	27
<b>15</b>	30.9 ± 1.1	10.5 ± 0.5	HepG2	48 h	27
<b>15</b>	50.3 ± 0.2	11.6 ± 0.2	HeLa	48 h	27
<b>16</b>	about 4	N/A	U373	72 h	31
<b>16</b>	about 4	N/A	MCF-7	72 h	31
<b>19</b>	78.2 ± 0.2	84	A2780/AD	24 h	29
<b>23c</b>	6.6 ± 3.0	42 ± 7 (5-FdU)	A549	48 h	35
<b>26d</b>	27 ± 11	42 ± 7 (5-FdU)	A549	48 h	50
<b>26e</b>	46 ± 22	42 ± 7 (5-FdU)	A549	48 h	50

<b>28</b>	3.4 ± 1.6	42 ± 7 (5-FdU)	A549	48 h	50
<b>29</b>	8.6 ± 0.2	2.7 ± 0.1	MCF-7	48 h	44
<b>29</b>	16.9 ± 1.5	8.2 ± 1.5	U2OS	48 h	44
<b>29</b>	29.8 ± 1.0	3.9 ± 1.0	HepG2	48 h	44
<b>30a</b>	13.6	19.5 (Ara-C)	HeLa	N/A	22
<b>30b</b>	11.9	19.5 (Ara-C)	HeLa	N/A	22
<b>30c</b>	11.4	19.5 (Ara-C)	HeLa	N/A	22
<b>30d</b>	8.6	19.5 (Ara-C)	HeLa	N/A	22
<b>30e</b>	9.9	19.5 (Ara-C)	HeLa	N/A	22
<b>31a</b>	6.4	19.5 (Ara-C)	HeLa	N/A	22
<b>31b</b>	5.7	19.5 (Ara-C)	HeLa	N/A	22
<b>31c</b>	6.5	19.5 (Ara-C)	HeLa	N/A	22
<b>32</b>	7.8	19.5 (Ara-C)	HeLa	N/A	22
<b>33</b>	5.2	51.2 (Ara-C)	MCF-7	N/A	24
<b>34</b>	3.4	51.2 (Ara-C)	MCF-7	N/A	24
<b>35, 36</b>	4-10	N/A	MCF-7	72 h	26
<b>35, 36</b>	4-10	N/A	EVSA-T	72 h	26
<b>35, 36</b>	4-10	N/A	NB69	72 h	26
<b>35, 36</b>	4-10	N/A	H4	72 h	26
<b>35, 36</b>	4-10	N/A	ECV	72 h	26
<b>37c</b>	7.3 ± 0.4	18 ± 1	MOLT-4	N/A	42
<b>37c</b>	24 ± 4	71 ± 8	MCF-7	N/A	42
<b>38a</b>	4.4	51.2 (Ara-C)	MCF-7	N/A	23
<b>38b</b>	2.6	51.2 (Ara-C)	MCF-7	N/A	23
<b>38c</b>	2.4	51.2 (Ara-C)	MCF-7	N/A	23
<b>38d</b>	2.6	51.2 (Ara-C)	MCF-7	N/A	23
<b>39</b>	1.0	51.2 (Ara-C)	MCF-7	N/A	23
<b>40b</b>	4.75	N/A	MCF-7	N/A	38
<b>40b</b>	75.1	N/A	HeLa	N/A	38
<b>17</b>	9.3 ± 2.2	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	66
<b>18</b>	17.3 ± 2.9	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	66
<b>47</b>	18.3 ± 1.4	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	66
<b>48</b>	5.4 ± 1.0	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	66
<b>48</b>	13.6 ± 1.7	N/A	PC-3	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	66
<b>49</b>	0.9 ± 0.1	N/A	HeLa	24 h 590–700 nm	46

				5 J cm <sup>-2</sup>	
<b>50</b>	3.3 ± 2.3	N/A	HeLa	24 h 590–700 nm 5 J cm <sup>-2</sup>	46
<b>51c</b>	0.1	N/A	HeLa	24 h >505 nm 105.1 J cm <sup>-2</sup>	43
<b>52</b>	9.3 ± 0.8	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	47
<b>53</b>	9.7 ± 4.4	9.2 ± 0.6	HeLa	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	47
<b>53</b>	19.2 ± 2.4	74.8 ± 14.8	PC-3	48 h 300–400 nm 2.6 J cm <sup>-2</sup>	47

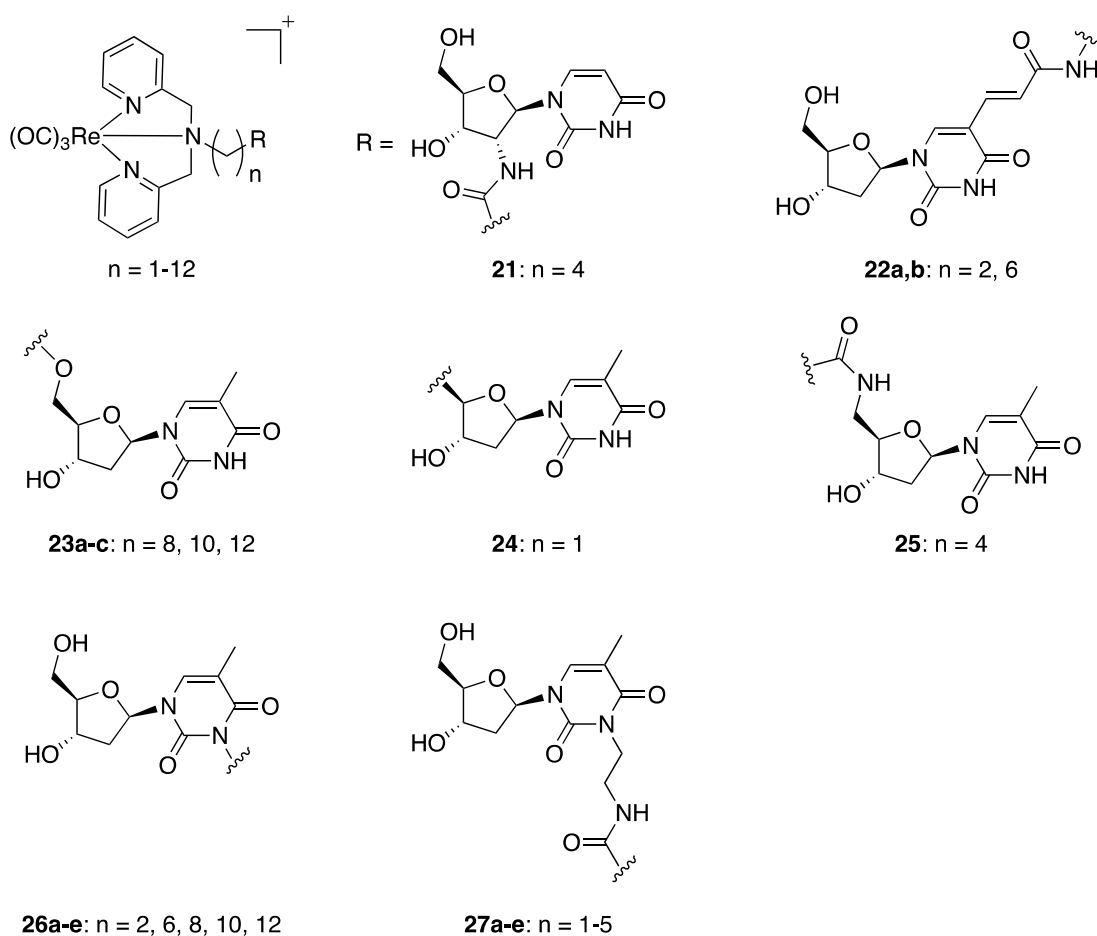
*a* – cisplatin unless otherwise indicated (5-FdU – 5-fluorodeoxyuridine, Ara-C – cytarabine), *N/A* – not available.

**Re(I) tricarbonyl complexes with *N, N, N*-tridentate ligands.** The second type of Re organometallic complexes that yielded several cytotoxic compounds is Re(I) tricarbonyl complexes with a tridentate ligand coordinated to the Re center *via* three N atoms (Figure 2). Dipicolylamine (Dpa) and *N, N*-bisquinoline (BQ) based ligands have been initially used with the Re(CO)<sub>3</sub> core for <sup>99m</sup>Tc/Re(I) studies and luminescent labeling. Specific targeting of the folate and cubilin receptors overexpressed in certain types of cancer has been achieved by coupling the [ReBQ(CO)<sub>3</sub>]<sup>+</sup> complexes **17** and **18** (Figure 2) to folate<sup>29</sup> and vitamin B<sub>12</sub>,<sup>34</sup> respectively. The resulting Re bioconjugates **19** and **20** (Figure 2) were rapidly taken up *via* the corresponding receptor-mediated pathways. The specificity of **19** and **20** was controlled by comparing the uptake on receptor overexpressing cell lines (human adramycin and cisplatin-resistant ovarian carcinoma A2780/AD for folate and human placental choriocarcinoma BeWo for B<sub>12</sub>) with that on a receptor-negative cell line (Chinese hamster ovary, CHO). In the case of the Re-folate conjugate **19**, fluorescence microscopy imaging revealed a rapid internalization of **19** by A2780/AD cells and a non-specific surface binding of **19** to CHO cells. Folate conjugation greatly increased the cytotoxicity of **17** from around 0.6 mM to 80 μM.<sup>29</sup> However, the non-specific binding of **19** to CHO cells resulted into the same anti-proliferative effect as on a folate receptor-positive A2780/AD cell line. Of note, a DNA-mediated mode of action for the Re-folate conjugate **19** could be ruled out. The biological activity of **19** has been attributed to the intact complex since 48 h incubation of **19** in PBS (pH 7.4) with a 100-fold excess of histidine or glutathione did not result in any change in LC-MS chromatogram. Fluorescence microscopy showed that the Re-B<sub>12</sub> conjugate **20**, on the other hand, displayed no emission in CHO cells and a strong signal in BeWo cells.<sup>34</sup> These results were reflected in the observed cytotoxicity of the Re-B<sub>12</sub> conjugate that was more active against the cubilin-positive BeWo cell line. Yet, compound **20** only produced its anti-proliferative effect at high concentration (IC<sub>50</sub> 2 mM).



staining by fluorescence microscopy.<sup>50</sup> While no exact <sup>99m</sup>Tc analogues of these complexes exist, several chemically robust <sup>99m</sup>Tc-labeled compounds have been prepared with dipicolylamine and *N, N*-bisquinoline ligands.<sup>67-71</sup> One of such derivatives has been used to image transplanted neural stem and progenitor cells in a SPECT/CT study on mice.<sup>68</sup>

As already discussed for Re bisimine compounds above, despite the favorable photophysical properties of certain Re organometallic complexes, emission microscopy localization is not always possible or accurate due to luminescence quenching. Complexes of type [ReBQ(CO)<sub>3</sub>]<sup>+</sup> sometimes share the same problem. For instance, a [ReBQ(CO)<sub>3</sub>]<sup>+</sup> complex with an azide group (**29** on Figure 2) could not be localized by fluorescence microscopy in HeLa cells, although the cytotoxicity profile indicates that it is taken up by cells. In fact, **29** approaches the cytotoxicity of cisplatin on U2OS, HepG2 and MCF-7 (IC<sub>50</sub> 6–39 μM, cisplatin 1–9 μM).<sup>44</sup> Although **29** could not be imaged in cells by fluorescent microscopy, the major mode of action of this [ReBQ(CO)<sub>3</sub>]<sup>+</sup> compound has been studied in detail using MCF-7 cells grown on a biosensor chip micro-bioreactor and isolated mitochondria. The complex had a strong effect on cellular metabolism by acting directly on mitochondria respiration. This activity was most likely due to the intact **29** as no decomposition was detected by LC-MS when **29** was incubated in human blood plasma for 72 h.

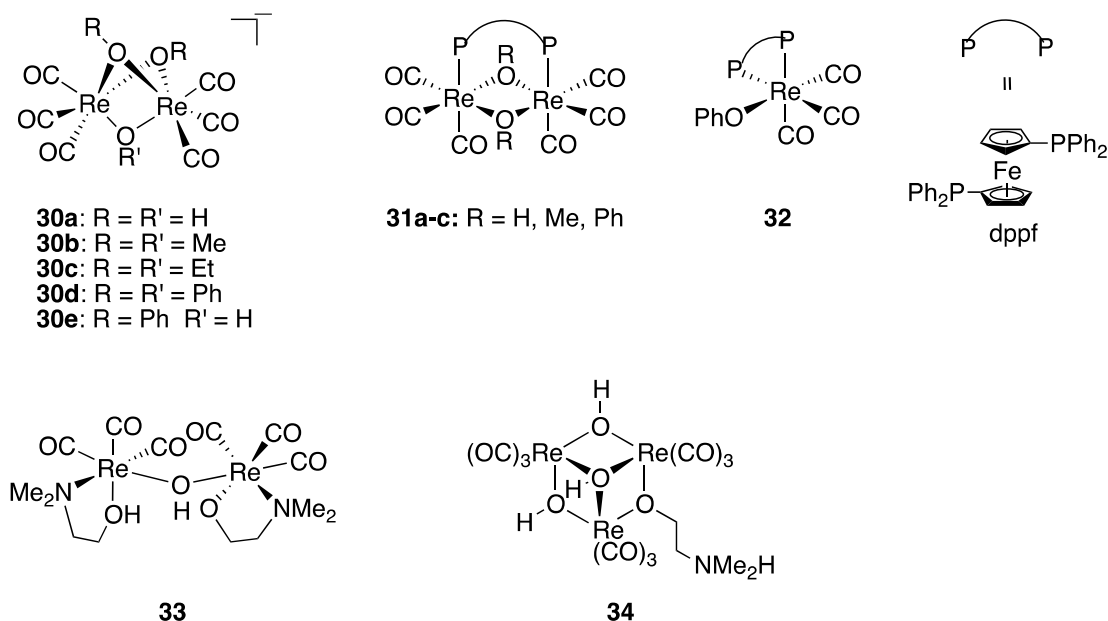


**Figure 3.** Cytotoxic Re(I) tricarbonyl complexes with dipicolylamine-based ligands.

**Alkoxo/hydroxo Re(I) carbonyl complexes.** Re(I) alkoxo/hydroxo carbonyl complexes (Figure 4) were the first Re organometallic compounds that were shown to possess an anti-cancer activity.<sup>22, 24</sup> Designed with the cisplatin ligand exchange-based mechanism of action in mind, this type of complexes has labile alkoxy/hydroxyl ligands. The dppf ligand of the Re compounds **31** and **32** (Figure 4) can also be released/exchanged and contributes to the overall cytotoxic effect, as dppf has been reported to be toxic on its own.<sup>72, 73</sup> Re(I) alkoxo/hydroxo compounds have been screened on over ten different human and murine tumor cell lines. These complexes were generally very active against suspended cancer cell cultures. However, on solid tumor cells, their cytotoxicity significantly varied with each particular cell line. The mechanism of toxicity has been described in considerable detail for compounds **30–31**.<sup>22</sup> The alkoxo/hydroxo Re(I)



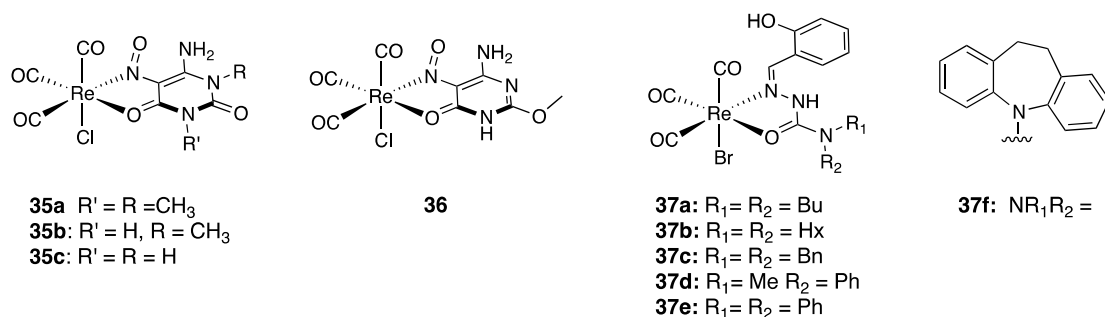
carbonyl compounds are able to cause DNA strand scission and severely affect the nucleic acid metabolism *via* enzymes such as dihydrofolate reductase, PRPP-amido transferase and thymidine kinase.<sup>22</sup> Similarly to **30–32**, complexes **33** and **34** (Figure 4) are more cytotoxic towards suspended cancer cells than solid tumor cell lines.<sup>24</sup> They, however, show improved activity on MCF-7 breast cancer. Their mechanism of action has not been studied, but their labile hydroxide and aminoethanol ligands are likely to be displaced in cells.



**Figure 4.** Cytotoxic Re(I) alcoxohydroxo complexes.

**Re(I) tricarbonyl complexes with NO bidentate ligand.** Two types of cytotoxic Re(I) carbonyl complexes coordinating a bidentate ligand *via* N and O atoms [Re(CO)<sub>3</sub>(N-O)X] (X = Cl, Br) have been reported to date (Figure 5).<sup>26, 42</sup> Various 5-nitrosopyrimidine Re complexes (**35**, **36**) induced a non-negligible cell growth at low concentration (up to 2  $\mu$ M), but reduced cellular proliferation at higher concentration (IC<sub>50</sub> 4–10  $\mu$ M) on five human tumor cell lines (breast cancer MCF-7 and EVSA-T, neuroblastoma NB69, glioma H4 and bladder carcinoma ECV).<sup>26</sup> Their mode of action was not explored, but enhanced cellular growth at lower concentration was explained in terms of the “heavy atom” effect on the estrogen receptor that has been previously described for Cd.<sup>74, 75</sup>

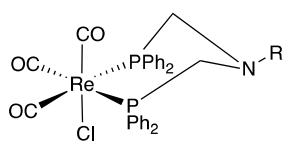
Semicarbazones were used as ligands in Re compounds **37**<sup>42</sup> since they are known to have their own pharmacological activity. The resulting complexes achieved cytotoxicity comparable to cisplatin towards acute lymphoblastic leukemia MOLT-4 cells ( $IC_{50}$  1–24  $\mu$ M, cisplatin 18  $\mu$ M) and did not strongly affect healthy fibroblast cells ( $IC_{50}$  > 125  $\mu$ M). The activity was correlated with lipophilicity and was generally similar or higher compared to non-coordinated semicarbazone ligands. However, most Re semicarbazone complexes displayed very low toxicity toward the MCF-7 cells. The “hit” Re semicarbazone compound **37c** that combined strong cytotoxicity (7.3 and 24  $\mu$ M) against both MOLT-4 and MCF-7 cancer cell lines with low toxicity towards healthy fibroblasts might have actually inherited its properties from the free ligand that has very similar  $IC_{50}$  values. Interestingly, NMR studies in DMSO showed that **37c** had a more acid phenolic proton than the corresponding ligand and tended to dimerize *via* loss of Br ligands. The authors judged dimerization in cell unlikely due to low concentrations, but hypothesized that good donor groups from biomolecules could easily substitute the Br ligand.



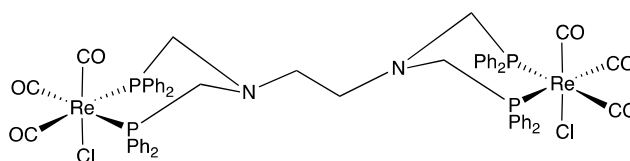
**Figure 5.** Cytotoxic Re(I) tricarbonyl complexes with N-O bidentate ligand.

**Re(I) tricarbonyl complexes with P-P and Se-Se bidentate ligands.** Tricarbonyl Re(I) bidentate phosphine complexes  $[Re(CO)_3(P-P)Br]$  **38** and **39** (Figure 6) have also been among the first Re organometallic compounds screened for cytotoxicity.<sup>23</sup> Tested on over 10 various cancer cell lines, these compounds were found to have  $IC_{50}$  values of about 5–10  $\mu$ M, showing particular activity against MCF-7 breast cancer and HeLa-S<sup>3</sup> suspended uterine carcinoma. More recently, Re(I) tricarbonyl complexes with diseleno ligands  $Re(CO)_3(Se-Se)Cl$  (**40**, **41** on Figure

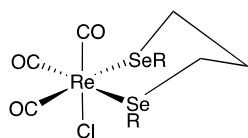
6) have been tested on HT 29 (colorectal cancer), MCF-7 (hormone-dependent breast cancer), A549S (lung adenocarcinoma) and HeLa (solid uterine carcinoma).<sup>38</sup> Curiously, these compounds also possessed a particularly high activity (4  $\mu$ M) against MCF-7 breast cancer cells, while being only moderately or essentially non-toxic to other cell lines. This property has been further explored in the course of an *in vivo* study on MCF-7 tumor bearing mice.<sup>41</sup> In this work, the treatment with Re organometallic complex **40b** was combined with cisplatin and Gd(III) administration, since both inorganic Re/cisplatin and Gd/cisplatin co-administration studies had previously shown synergetic action between cisplatin/Re and cisplatin/Gd(III) compounds, respectively. The intraperitoneal cisplatin administration (2 per week, 3 mg/kg) combined with oral daily (5/7 days/week) administration of a Gd complex ((tetrakis(1-octanol) tris(5-aminosalicylate) Gd(III)) and Re(CO)<sub>3</sub>(Se-Se)Cl (**40b**) led to 50% tumor volume reduction after 3 weeks treatment. By comparison, only 25% tumor volume reduction was observed for mice treated with cisplatin only. However, no control groups were treated with Re or Gd compound only, so the extent of complex **40b** contribution to tumor reduction has not been directly assessed. The largest effect on tumor volume was achieved using the maxim dose of Gd (100 mg/kg) and the minimal dose of Re (10 mg/kg). In the mice treated with higher doses of Re, more deaths were observed.



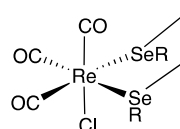
**38a-e:** R = Ph, CH<sub>2</sub>CH<sub>2</sub>OH, CH<sub>2</sub>COOCH<sub>2</sub>Ph, CH<sub>2</sub>CO-NHCH<sub>2</sub>COOCH<sub>2</sub>Ph, CH<sub>2</sub>COOH



**39**



**40a,b:** R = Ph, CH<sub>2</sub>COOH



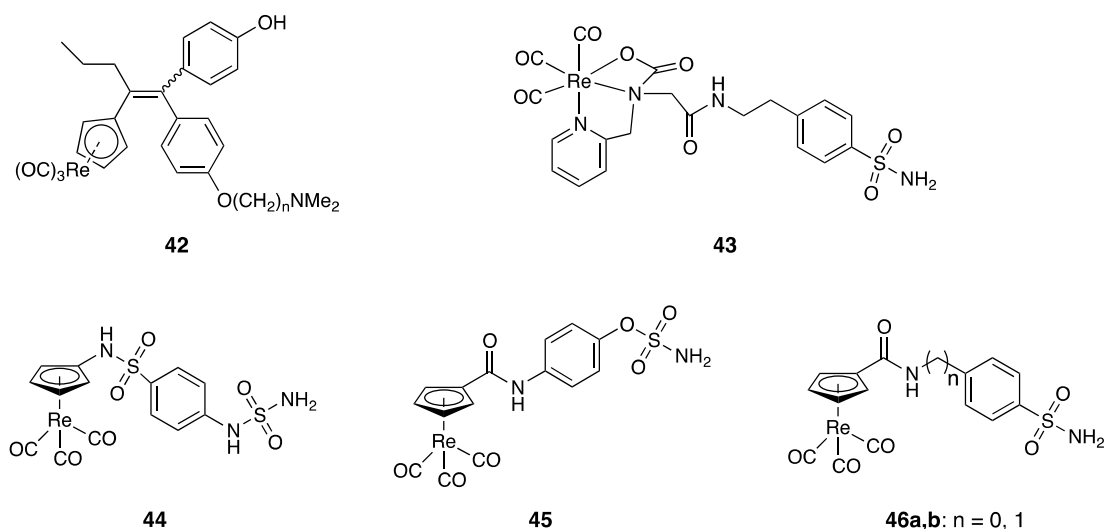
**41a,b:** R = Ph, CH<sub>2</sub>COOH

**Figure 6.** Cytotoxic Re(I) tricarbonyl complexes with P-P and Se-Se bidentate ligands.

**Re-containing enzyme and receptor inhibitors.** While a couple of cytotoxic Re complex discussed above might act *via* enzyme/receptor inhibition, none of them have been initially intended as such. In contrast, certain Re organometallic compounds, cyclopentadienyl (Cp) Re complexes in particular, have been purposely designed to bind a particular enzyme or receptor.<sup>25, 76, 77</sup> Specific interaction between Re complexes and their biological targets has been achieved by incorporating a  $\text{Re}(\text{CO})_3$  core into a known organic enzyme inhibitor or receptor ligand. The earliest examples of such Re compounds with anti-proliferative activity are Jaouen's Re-Tamoxifen (**42**, Figure 7) derivatives where a phenyl group is replaced by a Cp ring of  $[\text{Re}(\text{CO})_3\text{Cp}]$ .<sup>25</sup> Tamoxifen inhibits the estrogen receptor (ER) and is used for the treatment of hormone-responsive breast tumors. The derivatization with Re slightly decreased the affinity of Tamoxifen for ER. However, cytotoxicity assays on MCF-7 ER(+) breast cancer cells showed that Re-Tamoxifen conjugates were even slightly more active than Tamoxifen by itself. A small effect (about 10% reduction in cell viability at 1  $\mu\text{M}$ ) was also observed on Tamoxifen-resistant MDA-MB231 breast cancer cell line. *In vivo* distribution study in rats has been realized with a related compound where  $^{99\text{m}}\text{Tc}(\text{CO})_3\text{Cp}$  moiety was conjugated to atriaryl butane skeleton of tamoxifen *via* an amide bond.<sup>78</sup>

Recently, the  $\text{Re}(\text{CO})_3$  core has been conjugated to sulfonamide moieties to target human carbonic anhydrases (hCAs), which have emerged as an interesting target for therapy and diagnosis due to their overexpression induced by hypoxia in cancer cells.<sup>79-81</sup> High concentration of carbonic acid resulting from this excessive activity of hCAs leads to a pronounced decrease in extracellular pH (down to ca. 6 instead of 7.4).<sup>76, 79</sup> Re complexes (**43–46**, Figure 7) have been shown to be excellent hCA inhibitors with submicromolar affinities and interesting selectivity profiles for various hCA isoforms.<sup>76, 82</sup> No  $\text{IC}_{50}$  values were reported, but efficient inhibition of hCA in HeLa and HT29 cells has been demonstrated by monitoring the rise in extracellular pH upon incubation with Re inhibitors. The X-ray structure has shown that Re hCA inhibitors bound to the enzyme in a similar manner as organic inhibitors and the Re core only participated in

hydrophobic interactions with Phe, Leu and Pro amino acid residues.  $^{99m}\text{Tc}$  analogues of compounds **46a** and **b** have also been synthesized in an excellent yield,<sup>82</sup> while the  $^{99m}\text{Tc}$  version of inhibitor **43** has been already tested on xenograft mice with CAIX expressing tumors.<sup>76</sup> However, these *in vivo* biodistribution studies have shown that, despite excellent *in vitro* results, Re complex **43** failed to significantly accumulate in tumors.



**Figure 7.** Re organometallic enzyme and receptor inhibitors.

## PHOTO-TOXIC Re ORGANOMETALLIC COMPLEXES

Very few photo-toxic Re organometallic compounds have been identified to date (Figure 8).<sup>43, 44, 46, 47</sup> Most of them act as photodynamic therapy (PDT) photosensitizers (PS) – i.e. they transfer the energy received from light to oxygen (present in tissues) to generate reactive oxygen species (ROS). A PS excited by irradiation undergoes two types of reactions.<sup>83</sup> In the so-called type I reaction, the PS directly interacts with a substrate (e.g. cell membrane, protein) to form radicals or radical ions, which can in turn interact with oxygen to produce the highly reactive singlet oxygen. The type II reaction is defined as generation of singlet oxygen *via* PS-oxygen interaction.<sup>83</sup> Although many more Re compounds have been identified as efficient triplet

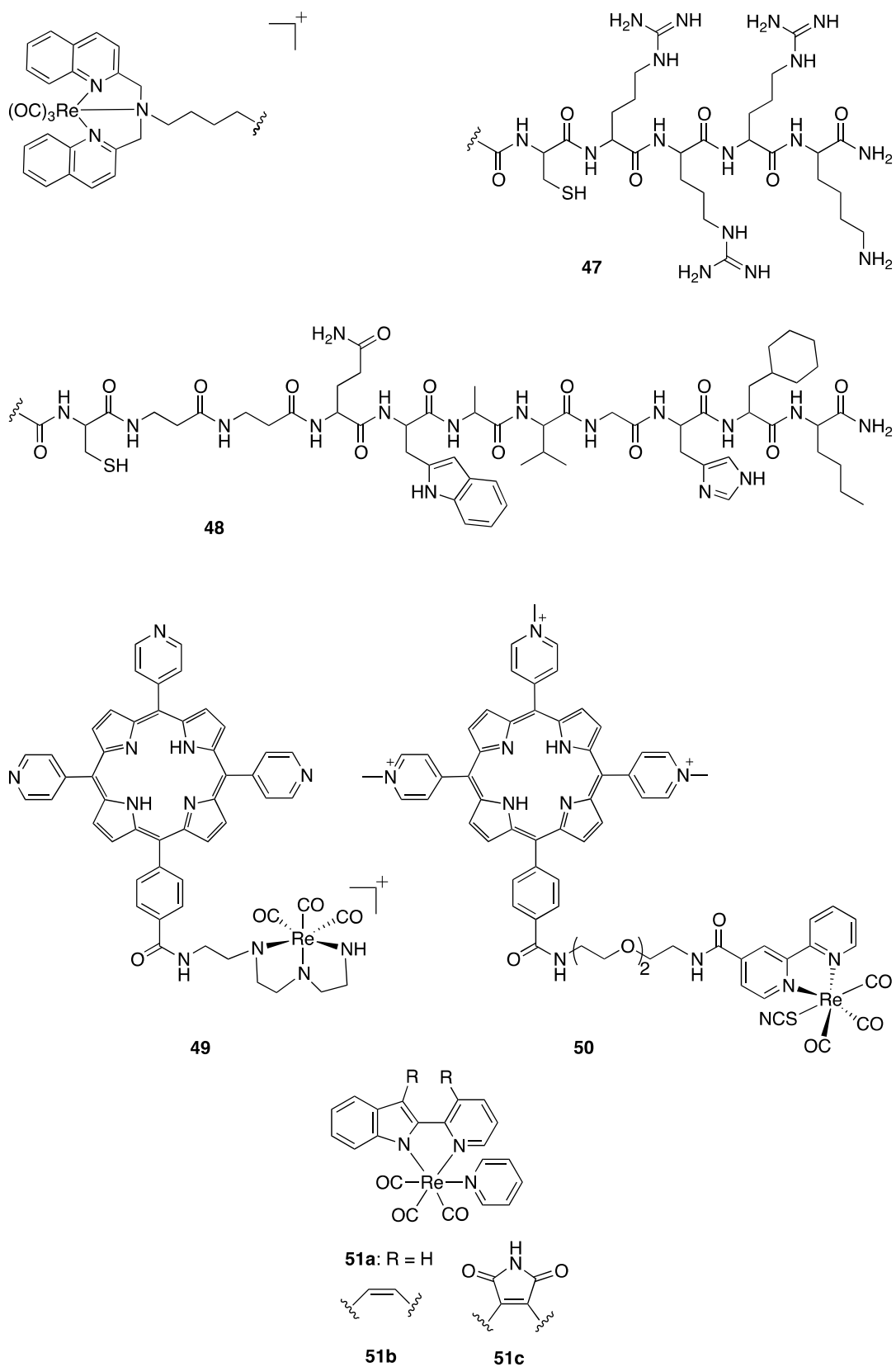
photosensitizers,<sup>39, 84-88</sup> this property has been more applied in photo-catalysis than in anti-cancer drug development.

The Re(I) tricarbonyl *N,N*-bisquinoline complexes [ReBQ(CO)<sub>3</sub>]<sup>+</sup> **17** and **18** already discussed above are capable of producing singlet oxygen when irradiated at 355 nm.<sup>44, 47</sup> Singlet oxygen generation of these Re complexes decreases with solvent polarity and therefore depends on their cellular localization. UV-A irradiation of the essentially non-toxic Re(I) tricarbonyl *N,N*-bisquinoline complexes **17** and **18** used in folate<sup>29</sup> and B<sub>12</sub><sup>34</sup> conjugation studies has revealed that the anti-proliferative activity of these compounds on HeLa cells could be increased by a 10-fold to 9.3 and 17.3 μM (HeLa) respectively by using a relatively low light dose (2.6 J/cm<sup>2</sup>).<sup>44</sup> This success, however, could not be reproduced on PC-3 cell line<sup>47</sup> without conjugation of the Re complex to a bombesin derivative (**48** on Figure 8) to target the GRP receptors overexpressed in certain types of cancer (5.4 μM on HeLa and 13.6 μM on PC-3).<sup>44, 47</sup> Addition of a nuclear localization signal peptide (NLS) (**47** on Figure 8) led to a strong increase of the dark cytotoxicity (35.1 μM HeLa). This could be the result of change in cellular localization upon NLS conjugation from cytoplasm to nucleoli, as observed by fluorescent microscopy. The bombesin derivative **48** could not be visualized on HeLa cells due to luminescence quenching.

Some photosensitizing porphyrin-Re conjugates (**49**, **50** on Figure 8) have been recently shown to greatly increase their cytotoxicity (from >100 μM to 3 μM IC<sub>50</sub>) upon 590–700 nm light irradiation.<sup>46</sup> However, the light activity of these compounds originated entirely from the porphyrin moiety as the Re complex present in the conjugate does not produce any singlet oxygen upon irradiation at 590–700 nm. The Re conjugation, however, was found to improve the uptake of these porphyrin-Re conjugates. Hot <sup>99m</sup>Tc analogues of **49** and **50** have also been prepared and shown to be stable in cell culture medium for at least 24 h by HPLC studies.<sup>89</sup>

Re organometallic compounds can also function as photosensitizers in the visible light range as recently demonstrated with Re(I) tricarbonyl indolato complexes (**51**).<sup>43</sup> These compounds' UV/vis absorption extends up to 600 nm so that visible light irradiation (>505 nm) results into up to a 1000 fold increase in anti-cancer activity on HeLa cells and the most active of them

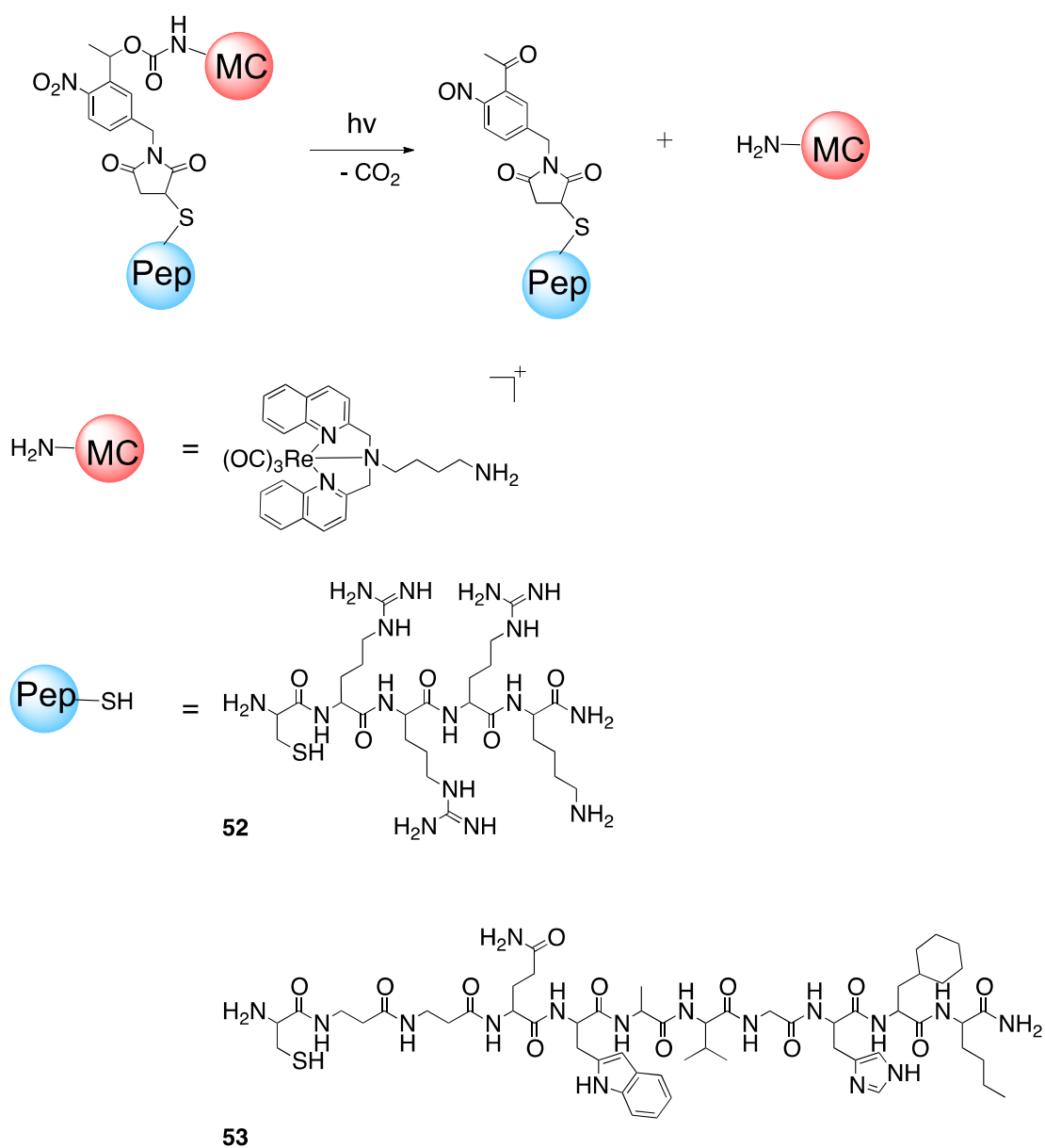
significantly reduced the size of melanoma spheroids. Singlet oxygen formation was detected for all complexes of the study by indirect *para*-nitrosodimethylaniline/imidazole assay<sup>90</sup> The most cytotoxic complex **51c** (0.1  $\mu$ M HeLa) was studied in more detail. Curiously, it underwent pyridine ligand substitution after irradiation in presence of 4-dimethylaminopyridine. Considerable ROS production was also detected *in vitro* after irradiation. Fluorescence and phase-contrast microscopy revealed that this complex was localizing in the cellular membrane and induced severe morphological changes in HeLa cells upon irradiation. In fact, addition of a membrane-localized anti-oxidant protected the cells from the anti-proliferative effect of the Re complex.



**Figure 8.** Photo-toxic Re organometallic compounds.



Recently, our group has incorporated a UVA (355 nm) photo-labile protecting group (PLPG) into the Re(I) *N,N*-bisquinoline bioconjugates **47** and **48** described above.<sup>47</sup> Upon irradiation at a very low light dose (1.2 J/cm<sup>2</sup>), the resulting photo-caged bioconjugates **52** and **53** (Scheme 1) liberated the Re complex **17** in addition to singlet oxygen generation. IC<sub>50</sub> values of c.a. 9 μM were thus reached. Biological studies demonstrated that the most cytotoxic compound **52** accumulated considerably in nucleoli where it could affect both DNA and RNA triggered cell death *via* both apoptosis and necrosis.



**Scheme 1.** Photo-caged Re organometallic compounds.

## CONCLUSIONS AND PERSPECTIVES

In this review, we have summarized the currently known Re organometallic compounds with anti-cancer activity, as well as highlighted their modes of cytotoxic action when possible. The majority of anti-proliferative Re organometallic complexes act as “traditional” chemotherapeutic agents, although a few photo-active Re complexes have also been recently reported. Potentially, an even larger number of Re organometallic compounds with PDT activity could be identified by screening the singlet oxygen production of known Re photocatalysts.

Most of the compounds presented in this review are potent anti-cancer agents with IC<sub>50</sub> values reaching or exceeding the activity of cisplatin. The underlying mechanisms of toxicity are, however, not always fully elucidated and, to the best of our knowledge, only one *in vivo* study has been conducted on cold Re organometallic complexes.<sup>41</sup> This study as well as a couple of successful *in vivo* evaluations of coordinative Re compounds<sup>91, 92</sup> have demonstrated a synergistic action of Re compounds with cisplatin. Undoubtedly, more thorough biological investigations both *in vitro* and *in vivo* would give us a better understanding of the cytotoxicity of Re organometallic complexes. In this context, preparation of “hot” analogues of promising Re compounds to visualize their *in vivo* behavior is an indispensable step and the most helpful tool to allow for the development of powerful and selective anti-cancer drugs.

## ACKNOWLEDGEMENTS

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## REFERENCES

- (1) Stewart, B. W., and Wild, C. P. (2014) World Cancer Report, World Health Organization.
- (2) (2011) Cancer survival statistics, Cancer Research UK, <http://www.cancerresearchuk.org/cancer-info/cancerstats/survival>.
- (3) Corrie, P. G. (2008) Cytotoxic chemotherapy: clinical aspects, *Medicine* 36, 24–28.
- (4) Jung, Y., and Lippard, S. J. (2007) Direct cellular responses to platinum-induced DNA damage, *Chem. Rev.* 107, 1387–1407.
- (5) Gasser, G., Ott, I., and Metzler-Nolte, N. (2010) Organometallic anticancer compounds, *J. Med. Chem.* 54, 3–25 and references therein.
- (6) Hartinger, C. G., Metzler-Nolte, N., and Dyson, P. J. (2012) Challenges and opportunities in the development of organometallic anticancer drugs, *Organometallics* 31, 5677–5685 and references therein.
- (7) Gasser, G., and Metzler-Nolte, N. (2012) The potential of organometallic complexes in medicinal chemistry, *Curr. Opin. Chem. Biol.* 16, 84–91 and references therein.
- (8) Bruijincx, P. C. A., and Sadler, P. J. (2008) New trends for metal complexes with anticancer activity, *Curr. Opin. Chem. Biol.* 12, 197–206 and references therein.

- (9) Hartinger, C. G., and Dyson, P. J. (2009) Bioorganometallic chemistry - from teaching paradigms to medicinal applications, *Chem. Soc. Rev.* **38**, 391–401 and references therein.
- (10) Jaouen, G., and Metzler-Nolte, N. (2010) *Medicinal organometallic chemistry*, Vol. 32, Springer-Verlag.
- (11) Peacock, A. F. A., and Sadler, P. J. (2008) Medicinal Organometallic Chemistry: Designing Metal Arene Complexes as Anticancer Agents, *Chem. Asian J.* **3**, 1890–1899 and references therein.
- (12) Smith, G. S., and Therrien, B. (2011) Targeted and multifunctional arene ruthenium chemotherapeutics, *Dalton Trans.* **40**, 10793–10800 and references therein.
- (13) Suss-Fink, G. (2010) Arene ruthenium complexes as anticancer agents, *Dalton Trans.* **39**, 1673–1688 and references therein.
- (14) Patra, M., and Gasser, G. (2012) Organometallic compounds, an opportunity for chemical biology?, *ChemBioChem* **13**, 1232–1252 and references therein.
- (15) Meggers, E. (2009) Targeting proteins with metal complexes, *Chem. Commun.*, 1001–1010.
- (16) Mulcahy, S., and Meggers, E. (2010) Organometallics as Structural Scaffolds for Enzyme Inhibitor Design, in *Medicinal Organometallic Chemistry* (Jaouen, G., and Metzler-Nolte, N., Eds.), pp 141–153, Springer Berlin Heidelberg.
- (17) Vessières, A., Top, S., Pigeon, P., Hillard, E., Boubeker, L., Spera, D., and Jaouen, G. (2005) Modification of the Estrogenic Properties of Diphenols by the Incorporation of Ferrocene. Generation of Antiproliferative Effects in Vitro, *J. Med. Chem.* **48**, 3937–3940.

- (18) Hillard, E., Vessières, A., Thouin, L., Jaouen, G., and Amatore, C. (2006) Ferrocene-Mediated Proton-Coupled Electron Transfer in a Series of Ferrocifen-Type Breast-Cancer Drug Candidates, *Angew. Chem. Int. Ed.* 45, 285–290.
- (19) Hamels, D., Dansette, P. M., Hillard, E. A., Top, S., Vessières, A., Herson, P., Jaouen, G., and Mansuy, D. (2009) Ferrocenyl Quinone Methides as Strong Antiproliferative Agents: Formation by Metabolic and Chemical Oxidation of Ferrocenyl Phenols, *Angew. Chem. Int. Ed.* 48, 9124–9126.
- (20) Pizarro, A. M., Habtemariam, A., and Sadler, P. J. (2010) Activation Mechanisms for Organometallic Anticancer Complexes, in *Medicinal Organometallic Chemistry* (Jaouen, G., and Metzler-Nolte, N., Eds.), pp 21–56, Springer Berlin Heidelberg.
- (21) Casini, A., Hartinger, C. G., Nazarov, A. A., and Dyson, P. J. (2010) Organometallic Antitumour Agents with Alternative Modes of Action, in *Medicinal Organometallic Chemistry* (Jaouen, G., and Metzler-Nolte, N., Eds.), pp 57–80, Springer Berlin Heidelberg.
- (22) Yan, Y. K., Cho, S. E., Shaffer, K. A., Rowell, J. E., Barnes, B. J., and Hall, I. H. (2000) Cytotoxicity of rhenium(I) alkoxo and hydroxo carbonyl complexes in murine and human tumor cells, *Pharmazie* 55, 307–313.
- (23) Zhang, J., Vittal, J. J., Henderson, W., Wheaton, J. R., Hall, I. H., Hor, T. S. A., and Yan, Y. K. (2002) Tricarbonylrhenium(I) complexes of phosphine-derivatized amines, amino acids and a model peptide: structures, solution behavior and cytotoxicity, *J. Organomet. Chem.* 650, 123–132.

- (24) Wang, W., Yan, Y. K., Hor, T. S. A., Vittal, J. J., Wheaton, J. R., and Hall, I. H. (2002) Synthesis, X-ray structures, and cytotoxicity of rhenium(I) carbonyl 2-(dimethylamino)ethoxide complexes, *Polyhedron* 21, 1991–1999.
- (25) Vessieres, A., Rager, M. N., Huche, M., Top, S., Vessièrès, A., Pigeon, P., Rager, M.-N., Huché, M., Salomon, E., Cabestaing, C., Vaissermann, J., and Jaouen, G. (2004) Selective Estrogen-Receptor Modulators (SERMs) in the Cyclopentadienylrhenium Tricarbonyl Series: Synthesis and Biological Behaviour, *ChemBioChem* 5, 1104–1113.
- (26) Illán-Cabeza, N. A., García-García, A. R., Moreno-Carretero, M. N., Martínez-Martos, J. M., and Ramírez-Expósito, M. J. (2005) Synthesis, characterization and antiproliferative behavior of tricarbonyl complexes of rhenium(I) with some 6-amino-5-nitrosouracil derivatives: Crystal structure of fac-[ReCl(CO)<sub>3</sub>(DANU-N5,O4)] (DANU = 6-amino-1,3-dimethyl-5-nitrosouracil), *J. Inorg. Biochem.* 99, 1637–1645.
- (27) Ma, D.-L., Che, C.-M., Siu, F.-M., Yang, M., and Wong, K.-Y. (2007) DNA Binding and Cytotoxicity of Ruthenium(II) and Rhenium(I) Complexes of 2-Amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine, *Inorg. Chem.* 46, 740–749.
- (28) Lo, K. K.-W., Louie, M.-W., Sze, K.-S., and Lau, J. S.-Y. (2007) Rhenium(I) Polypyridine Biotin Isothiocyanate Complexes as the First Luminescent Biotinylation Reagents: Synthesis, Photophysical Properties, Biological Labeling, Cytotoxicity, and Imaging Studies, *Inorg. Chem.* 47, 602–611.
- (29) Viola-Villegas, N., Rabideau, A. E., Cesnavicious, J., Zubieta, J., and Doyle, R. P. (2008) Targeting the folate receptor (FR): imaging and cytotoxicity of Re(I) conjugates in FR-overexpressing cancer cells, *ChemMedChem* 3, 1387–1394.

- (30) Orsa, D. K., Haynes, G. K., Pramanik, S. K., Iwunze, M. O., Greco, G. E., Ho, D. M., Krause, J. A., Hill, D. A., Williams, R. J., and Mandal, S. K. (2008) The one-pot synthesis and the fluorescence and cytotoxicity studies of chlorotricarbonyl( $\alpha$ -diimine)rhenium(I), fac-(CO)<sub>3</sub>( $\alpha$ -diimine)ReCl, complexes, *Inorg. Chem. Commun.* **11**, 1054–1056.
- (31) Picon-Ferrer, I., Hueso-Urena, F., Illan-Cabeza, N. A., Jimenez-Pulido, S. B., Martinez-Martos, J. M., Ramirez-Exposito, M. J., and Moreno-Carretero, M. N. (2009) Chloro-fac-tricarbonylrhenium(I) complexes of asymmetric azines derived from 6-acetyl-1,3,7-trimethylpteridine-2,4(1H,3H)-dione with hydrazine and aromatic aldehydes: Preparation, structural characterization and biological activity against several human tumor cell lines, *J. Inorg. Biochem.* **103**, 94–100.
- (32) Louie, M.-W., Liu, H.-W., Lam, M. H.-C., Lau, T.-C., and Lo, K. K.-W. (2009) Novel Luminescent Tricarbonylrhenium(I) Polypyridine Tyramine-Derived Dipicolylamine Complexes as Sensors for Zinc(II) and Cadmium(II) ions, *Organometallics* **28**, 4297–4307.
- (33) Louie, M.-W., Ho-Chuen Lam, K., and Kam-Wing, L. (2009) Luminescent Polypyridinerhenium(I) Bis-Biotin Complexes as Crosslinkers for Avidin, *Eur. J. Inorg. Chem.* **2009**, 4265–4273.
- (34) Viola-Villegas, N., Rabideau, A. E., Bartholoma, M., Zubieta, J., and Doyle, R. P. (2009) Targeting the cubilin receptor through the vitamin B12 uptake pathway: cytotoxicity and mechanistic insight through fluorescent Re(I) delivery, *J. Med. Chem.* **52**, 5253–5261.
- (35) Bartholoma, M. D., Vortherms, A. R., Hillier, S., Ploier, B., Joyal, J., Babich, J., Doyle, R. P., and Zubieta, J. (2010) Synthesis, cytotoxicity, and insight into

- the mode of action of  $\text{Re}(\text{CO})_3$  thymidine complexes, *ChemMedChem* 5, 1513–1529.
- (36) Fernandez-Moreira, V., Thorp-Greenwood, F. L., Amoroso, A. J., Cable, J., Court, J. B., Gray, V., Hayes, A. J., Jenkins, R. L., Kariuki, B. M., Lloyd, D., Millet, C. O., Williams, C. F., and Coogan, M. P. (2010) Uptake and localisation of rhenium fac-tricarbonyl polypyridyls in fluorescent cell imaging experiments, *Org. Biomol. Chem.* 8, 3888–3901.
- (37) Lo, K. K.-W., Zhang, K. Y., and Li, S. P.-Y. (2011) Recent exploitation of luminescent rhenium(I) tricarbonyl polypyridine complexes as biomolecular and cellular probes, *Eur. J. Inorg. Chem.*, 3551–3568.
- (38) Kermagoret, A., Morgant, G., d'Angelo, J., Tomas, A., Roussel, P., Bastian, G., Collery, P., and Desmaële, D. (2011) Synthesis, structural characterization and biological activity against several human tumor cell lines of four rhenium(I) diseleno-ethers complexes:  $\text{Re}(\text{CO})_3\text{Cl}(\text{PhSe}(\text{CH}_2)_2\text{SePh})$ ,  $\text{Re}(\text{CO})_3\text{Cl}(\text{PhSe}(\text{CH}_2)_3\text{SePh})$ ,  $\text{Re}(\text{CO})_3\text{Cl}(\text{HO}_2\text{C}-\text{CH}_2\text{Se}(\text{CH}_2)_2\text{SeCH}_2-\text{CO}_2\text{H})$  and  $\text{Re}(\text{CO})_3\text{Cl}(\text{HO}_2\text{C}-\text{CH}_2\text{Se}(\text{CH}_2)_3\text{SeCH}_2-\text{CO}_2\text{H})$ , *Polyhedron* 30, 347–353.
- (39) Choi, A. W.-T., Louie, M.-W., Li, S. P.-Y., Liu, H.-W., Chan, B. T.-N., Lam, T. C.-Y., Lin, A. C.-C., Cheng, S.-H., and Lo, K. K.-W. (2012) Emissive behavior, cytotoxic activity, cellular uptake, and pegylation properties of new luminescent rhenium(I) polypyridine poly(ethylene glycol) complexes, *Inorg. Chem.* 51, 13289–13302.
- (40) Mbagu, M. K., Kebulu, D. N., Winstead, A., Pramanik, S. K., Banerjee, H. N., Iwunze, M. O., Wachira, J. M., Greco, G. E., Haynes, G. K., Sehmer, A., Sarkar, F. H., Ho, D. M., Pike, R. D., and Mandal, S. K. (2012) Fac-tricarbonyl(pentylcarbonato)( $\alpha$ -diimine)rhenium complexes: One-pot



- synthesis, characterization, fluorescence studies, and cytotoxic activity against human MDA-MB-231 breast, CCL-227 colon and BxPC-3 pancreatic carcinoma cell lines, *Inorg. Chem. Commun.* **21**, 35–38.
- (41) Collery, P., Mohsen, A., Kermagoret, A., D'Angelo, J., Morgant, G., Desmaële, D., Tomas, A., Collery, T., Wei, M., and Badawi, A. (2012) Combination of three metals for the treatment of cancer: gallium, rhenium and platinum. 1. Determination of the optimal schedule of treatment, *Anticancer Res.* **32**, 2769–2781.
- (42) Ho, J., Lee, W. Y., Koh, K. J. T., Lee, P. P. F., and Yan, Y.-K. (2013) Rhenium(I) tricarbonyl complexes of salicylaldehyde semicarbazones: synthesis, crystal structures and cytotoxicity, *J. Inorg. Biochem.* **119**, 10–20.
- (43) Kastl, A., Dieckmann, S., Wähler, K., Völker, T., Kastl, L., Merkel, A. L., Vultur, A., Shannan, B., Harms, K., Ocker, M., Parak, W. J., Herlyn, M., and Meggers, E. (2013) Rhenium complexes with visible-light-induced anticancer activity, *ChemMedChem* **8**, 924–927.
- (44) Kitanovic, I., Can, S., Alborzinia, H., Kitanovic, A., Pierroz, V., Leonidova, A., Pinto, A., Spingler, B., Ferrari, S., Molteni, R., Steffen, A., Metzler-Nolte, N., Wolfl, S., and Gasser, G. (2013) Photophysical, theoretical and biological evaluation and cell death mechanisms of a Re(I) organometallic complex, *Chem. Eur. J.* **20**, 2496–2507.
- (45) Parson, C., Smith, V., Krauss, C., Banerjee, H. N., Reilly, C., Krause, J. A., Wachira, J. M., Giri, D., Winstead, A., and Mandal, S. K. (2014) Anticancer Properties of Novel Rhenium Pentylcarbanato Compounds against MDA-MB-468(HTB-132) Triple Node Negative Human Breast Cancer Cell Lines, *Brit. J. Pharm. Res.* **4**, 362–367.

- (46) Gianferrara, T., Spagnul, C., Alberto, R., Gasser, G., Ferrari, S., Pierroz, V., Bergamo, A., and Alessio, E. (2014) Towards matched pairs of porphyrin–ReI/<sup>99m</sup>TcI conjugates that combine photodynamic activity with fluorescence and radio imaging, *ChemMedChem* 9, 1231–1237.
- (47) Leonidova, A., Pierroz, V., Rubbiani, R., Lan, Y., Schmitz, A. G., Kaech, A., Sigel, R. K. O., Ferrari, S., and Gasser, G. (2014) Photo-induced uncaging of a specific Re(I) organometallic complex in living cells, *Chem. Sci.*, 10.1039/C1033SC53550A.
- (48) Thorp-Greenwood, F. L., Balasingham, R. G., and Coogan, M. P. (2012) Organometallic complexes of transition metals in luminescent cell imaging applications *J. Organomet. Chem.* 714, 12–21.
- (49) Amoroso, A. J., Coogan, M. P., Dunne, J. E., Fernandez-Moreira, V., Hess, J. B., Hayes, A. J., Lloyd, D., Millet, C., Pope, S. J. A., and Williams, C. (2007) Rhenium fac tricarbonyl bisimine complexes: biologically useful fluorochromes for cell imaging applications, *Chem. Commun.* 29, 3066–3068.
- (50) Bartholoma, M. D., Vortherms, A. R., Hillier, S., Joyal, J., Babich, J., Doyle, R. P., and Zubieta, J. (2011) Synthesis, cytotoxicity and cellular uptake studies of N3 functionalized Re(CO)<sub>3</sub> thymidine complexes, *Dalton Trans.* 40, 6216–6225.
- (51) Leonidova, A., Pierroz, V., Adams, L. A., Barlow, N., Ferrari, S., Graham, B., and Gasser, G. (2014) Enhanced Cytotoxicity through Conjugation of a “Clickable” Luminescent Re(I) Complex to a Cell-Penetrating Lipopeptide, *ACS Med. Chem. Lett.* 5, 809–814.
- (52) Dilworth, J. (1998) The biomedical chemistry of technetium and rhenium, *Chem. Soc. Rev.* 27, 43.

- (53) Coogan, M., Coogan, R., Doyle, J., Valliant, J., Babich, J., and Zubieta. (2014) Single amino acid chelate complexes of the  $M(CO)_3^+$  core for correlating fluorescence and radioimaging studies ( $M = {}^{99m}\text{Tc}$  or  $\text{Re}$ ), *J. Labelled Comp. Radiopharm.* 57, 255–261.
- (54) Jürgens, S., Herrmann, W. A., and Kühn, F. E. (2014) Rhenium and technetium based radiopharmaceuticals: Development and recent advances, *J. Organomet. Chem.* 751, 83–89.
- (55) Kluba, C., and Mindt, T. (2013) Click-to-chelate: development of technetium and rhenium-tricarbonyl labeled radiopharmaceuticals, *Molecules* 18, 3206–3226.
- (56) Knapp, F. F. J. (1998) Rhenium-188 - A Generator-Derived Radioisotope for Cancer Therapy, *Cancer Biother. Radiopharm.* 13, 337–349.
- (57) Garcia-Garayoa, E., Schibli, R., and Schubiger, P. (2007) Peptides radiolabeled with  $\text{Re-186/188}$  and  $\text{Tc-99m}$  as potential diagnostic and therapeutic agents, *Nucl. Sci. Tech.* 18, 88–100.
- (58) Boschi, A., Uccelli, L., Pasquali, M., Duatti, A., Taibi, A., Pupillo, G., and Esposito, J. (2014)  $188\text{W}/188\text{Re}$  Generator System and Its Therapeutic Applications, *J. Chem.*, <http://dx.doi.org/10.1155/2014/529406>.
- (59) Pillai, M. R., Dash, A., and Knapp, F. F. J. (2012) Rhenium-188: Availability from the  $188\text{W}/188\text{Re}$  Generator and Status of Current Applications, *Curr. Radiopharm.* 5, 222–243.
- (60) Gasser, G., Neumann, S., Ott, I., Seitz, M., Heumann, R., and Metzler-Nolte, N. (2011) Preparation and Biological Evaluation of Di-Hetero Organometallic-Containing PNA Bioconjugates., *Eur. J. Inorg. Chem.* 36, 5471–5478.

- (61) Pitchumony, T. S., Banevicius, L., Janzen, N., Zubieta, J., and Valliant, J. F. (2013) Isostructural Nuclear and Luminescent Probes Derived From Stabilized [2 + 1] Rhenium(I)/Technetium(I) Organometallic Complexes, *Inorg. Chem.* 52, 13521–13528.
- (62) Gottschaldt, M., Koth, D., Müller, D., Klette, I., Rau, S., Görls, H., Schäfer, B., Baum, R. P., and Yano, S. (2007) Synthesis and structure of novel sugar-substituted bipyridine complexes of rhenium and <sup>99m</sup>technetium, *Chem. Eur. J.* 13, 10273–10280.
- (63) Zhang, X., and Chen, X. (2007) Preparation and characterization of <sup>99m</sup>Tc(CO)<sub>3</sub>-BPy-RGD complex as alpha v beta-3 integrin receptor-targeted imaging agent, *Appl. Radiat. Isotopes* 65, 70-78.
- (64) Alberto, R. (2009) The chemistry of technetium–water complexes within the manganese triad: challenges and perspectives, *Eur. J. Inorg. Chem.*, 21-31.
- (65) Louie, M.-W., Choi, A. W.-T., Liu, H.-W., Chan, B. T.-N., and Lo, K. K.-W. (2012) Synthesis, emission characteristics, cellular studies, and bioconjugation properties of luminescent rhenium(I) polypyridine complexes with a fluororous pendant, *Organometallics* 31, 5844-5855.
- (66) Leonidova, A., Pierroz, V., Rubbiani, R., Heier, J., Ferrari, S., and Gasser, G. (2013) Towards Cancer Cell-Specific Phototoxic Organometallic Rhenium(I) Complexes, *Dalton Trans.* 43, 4287–4294
- (67) Banerjee, S. R., Schaffer, P., Babich, J. W., Valliant, J. F., and Zubieta, J. (2005) Design and synthesis of site directed maleimide bifunctional chelators for technetium and rhenium, *Dalton Trans.*, 3886-3897.
- (68) Schaffer, P., Gleave, J. A., Lemon, J. A., Reid, L. C., Pacey, L. K. K., Farncombe, T. H., Boreham, D. R., Zubieta, J., Babich, J. W., Doering, L. C., and Valliant, J. F.

- (2008) Isostructural fluorescent and radioactive probes for monitoring neural stem and progenitor cell transplants, *Nucl. Med. Biol.* 35, 159-169.
- (69) Stephenson, K. A., Reid, L. C., Zubieta, J., Babich, J. W., Kung, M.-P., Kung, H. F., and Valliant, J. F. (2008) Synthesis and Screening of a Library of Re/Tc-Based Amyloid Probes Derived from  $\alpha$ -Breaker Peptides, *Bioconjugate Chem.* 19, 1087-1094.
- (70) Stephenson, K. A., Banerjee, S. R., Besanger, T., Sogbein, O. O., Levadala, M. K., McFarlane, N., Lemon, J. A., Boreham, D. R., Maresca, K. P., Brennan, J. D., Babich, J. W., Zubieta, J., and Valliant, J. F. (2004) Bridging the Gap between in Vitro and in Vivo Imaging: Isostructural Re and  $^{99m}\text{Tc}$  Complexes for Correlating Fluorescence and Radioimaging Studies, *J. Am. Chem. Soc.* 126, 8598-8599.
- (71) James, S., Maresca, K. P., Babich, J. W., Valliant, J. F., Doering, L., and Zubieta, J. (2006) Isostructural Re and  $^{99m}\text{Tc}$  Complexes of Biotin Derivatives for Fluorescence and Radioimaging Studies, *Bioconjugate Chem.* 17, 590-596.
- (72) Scarcia, V., Furlani, A., Longato, B., Corain, B., and Pilloni, G. (1988) Heteropolymetallic complexes of 1,1'-bis(diphenylphosphino)ferrocene (dppf). IV. Solvolytic behavior and cytostatic properties towards the KB cell-line of dppf and 1,2-bis(diphenylphosphino)ethane cis-complexes of Pt(II) and Pd(II), *Inorg. Chim. Acta* 153, 67-70.
- (73) Hill, D. T., Girard, G. R., McGabe, F. L., Johnson, R. K., Stupik, P. D., Zhang, J. H., Reiff, W. M., and Eggleston, D. S. (1989) [p- l,l'-Bis(diphenylphosphino)ferrocene]bis(chlorogold): Synthesis, Iron-57 and gold-197 mossbauer spectroscopy, x-ray crystal structure, and antitumor activity *Inorg. Chem.* 28, 3529-3533.

- (74) Choe, S.-Y., Kim, H. G., Lee, J. H., Choi, Y., Lee, H., and Kim, Y. (2003) Evaluation of estrogenicity of major heavy metals, *Sci. Tot. Environ.* 312, 15-21.
- (75) Safe, S. (2003) Cadmium's disguise dupes the estrogen receptor, *Nat. Med.* 9, 1000-1001.
- (76) Akurathi, V., Dubois, L., Lieuwes, N. G., Chitneni, S. K., Cleynhens, B. J., Vullo, D., Supuran, C. T., Verbruggen, A. M., Lambin, P., and Bormans, G. M. (2010) Synthesis and biological evaluation of a <sup>99m</sup>Tc-labelled sulfonamide conjugate for in vivo visualization of carbonic anhydrase IX expression in tumor hypoxia, *Nucl. Med. Biol.* 37, 557–564.
- (77) Agasti, S. S., Chompoosor, A., You, C.-C., Ghosh, P., Kim, C. K., and Rotello, V. M. (2009) Photoregulated Release of Caged Anticancer Drugs from Gold Nanoparticles, *J. Am. Chem. Soc.* 131, 5728–5729.
- (78) Dallagi, T., Saidi, M., Vessièrès, A., Huché, M., Jaouen, G., and Top, S. (2013) Synthesis and antiproliferative evaluation of ferrocenyl and cymantrenyl triaryl butene on breast cancer cells. Biodistribution study of the corresponding technetium-99m tamoxifen conjugate, *J. Organomet. Chem.* 734, 69–77.
- (79) Supuran, C. T. (2008) Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, *Nat. Rev. Drug Discov.* 7, 168–181.
- (80) Potter, C. P. S., and Harris, A. L. (2003) Diagnostic, prognostic and therapeutic implications of carbonic anhydrases in cancer, *Brit. J. Cancer* 89, 2–7.
- (81) Sung, F. L., Hui, E. P., Tao, Q., Li, H., Tsui, N. B. Y., Dennis Lo, Y. M., Ma, B. B. Y., To, K. F., Harris, A. L., and Chan, A. T. C. (2007) Genome-wide expression analysis using microarray identified complex signaling pathways

- modulated by hypoxia in nasopharyngeal carcinoma, *Cancer Lett.* 253, 74–88.
- (82) Can, D., Spingler, B., Schmutz, P., Mendes, F., Raposinho, P., Fernandes, C., Carta, F., Innocenti, A., Santos, I., Supuran, C. T., and Alberto, R. (2012) [(Cp-R)M(CO)<sub>3</sub>] (M=Re or <sup>99m</sup>Tc) Arylsulfonamide, Arylsulfamide, and Arylsulfamate Conjugates for Selective Targeting of Human Carbonic Anhydrase IX, *Angew. Chem.* 124, 3410–3413.
- (83) Dolmans, D. E. J. G. J., Fukumura, D., and Jain, R. K. (2003) Photodynamic Therapy for Cancer, *Nat. Rev. Cancer* 3, 380–387 and the references therein.
- (84) Gao, Y., Sun, S., and Han, K. (2009) Electronic structures and spectroscopic properties of rhenium (I) tricarbonyl photosensitizer: [Re(4,4'-(COOEt)<sub>2</sub>-2,2'-bpy)(CO)<sub>3</sub>py]PF<sub>6</sub>, *Spectrochim. Acta A* 71, 2016-2022.
- (85) Liu, J., and Jiang, W. (2012) Photoinduced hydrogen evolution in supramolecular devices with a rhenium photosensitizer linked to FeFe-hydrogenase model complexes, *Dalton Trans.* 41, 9700-9707.
- (86) Abdel-Shafi, A. A., Bourdelandeb, J. L., and Ali, S. S. (2007) Photosensitized generation of singlet oxygen from rhenium(I) and iridium(III) complexes, *Dalton Trans.* 24, 2510–2516.
- (87) Probst, B., Rodenberg, A., Guttentag, M., Hamm, P., and Alberto, R. (2010) A highly stable rhenium-cobalt system for photocatalytic H<sub>2</sub> production: unraveling the performance-limiting steps, *Inorg. Chem.* 49, 6453–6460.
- (88) Probst, B., Guttentag, M., Rodenberg, A., Hamm, P., and Alberto, R. (2011) Photocatalytic H<sub>2</sub> production from water with rhenium and cobalt complexes, *Inorg. Chem.* 50, 3404–3412.

- (89) Spagnul, C., Alberto, R., Gasser, G., Ferrari, S., Pierroz, V., Bergamo, A., Gianferrara, T., and Alessio, E. (2013) Novel water-soluble  $^{99\text{m}}\text{Tc}(\text{I})/\text{Re}(\text{I})$ -porphyrin conjugates as potential multimodal agents for molecular imaging, *J. Inorg. Biochem.* 122, 57–65.
- (90) Kraljić, I., and Mohsni, S. E. (1978) A new method for the detection of singlet oxygen in aqueous solutions, *Photochem. Photobiol.* 28, 577–581.
- (91) Shtemenko, A. V., Collery, P., Shtemenko, N. I., Domasevitch, K. V., Zabitskaya, E. D., and Golichenko, A. A. (2009) Synthesis, characterization, in vivo antitumor properties of the cluster rhenium compound with GABA ligands and its synergism with cisplatin, *Dalton Trans.*, 5132–5136.
- (92) Shtemenko, N., Collery, P., and Shtemenko, A. (2007) Dichlorotetra-  $\mu$  - Isobutyratodirhenium(III): Enhancement of cisplatin action and RBC-stabilizing Properties, *Anticancer Res.* 27, 2487–2492.